U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20090-3630

CHAPTER 12

VICAM - DON FQ TEST KIT

12.1 TESTING AREA

The extraction solution and other materials used in the DON FQ test kit necessitates the use of separate FGIS-approved laboratory space. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

12.2 EXTRACTION PROCEDURES

a. Preparation of Extraction Solvent (84 Percent Acetonitrile Solution).

Make up the solution by using the ratio of 84 parts acetonitrile to 16 parts deionized/distilled water. Prepare the 84 percent acetonitrile solution by adding 840 ml acetonitrile to 160 ml of distilled or deionized water. Mix well. Label the solution bottle and keep it tightly capped when not in use.

If the amount of solution being prepared needs to be adjusted based on the workload at individual locations, make sure that 84 parts acetonitrile to 16 parts deionized/distilled water ratio is maintained.

b. Extraction Procedures.

(1) Place 50 grams of ground sample into a blender jar.

(2) Add 200 ml of acetonitrile/water (84/16) and blend on high for 3 minutes.

(3) Filter into a sample container using coffee filters or Whatman No. 1 filter paper.

12.3 TEST PROCEDURES

a. Purification Procedures.

NOTE: All solution transfers may be carried out using adjustable automatic pipettors with disposable tips. Care should be taken to make sure that the tips used are large enough to hold the volume being transferred. Make sure that they are securely attached to the pipettor.

(1) Place 4 ml of extract in a 15 x 85 culture tube. Insert a MycoSep #225 column into the top of the culture tube and slowly (20 seconds) push to the bottom of the tube.

(Note: Use 6 ml of extract and a MycoSep #227 column for malted barley samples and take 30 seconds to push the extract through the column.)

(2) Transfer 1.5 ml of each purified sample extract to a 12 x 75 mm cuvette. Use a clean pipette tip for each transfer.

b. Calibrators and Control Preparation.

(1) Allow the calibrator and control solutions to come to room temperature.

(2) Invert each calibrator standard bottle and control standard bottle three times to mix thoroughly.

(3) Transfer 1.5 ml of the green-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(4) Using a clean tip, transfer 1.5 ml of the red-labeled calibrator solution to a clean 12 x 75 mm cuvette.

(5) Using a clean tip, transfer 1.5 ml of the control (yellow-labeled) solution to a clean 12 x 75 mm cuvette.

(6) Cap the calibrator solutions tightly and store in the refrigerator.

(7) Proceed with the analysis, treating samples, calibrators, and control identically.

c. Evaporation Procedures.

Evaporate each sample, calibrator, and control to dryness using a vacuum manifold and dry bath set at 70°C.

Note: To decrease the evaporation time, turn off the vacuum to the manifold for the rows that are not being used.

d. Derivatization Procedures.

(1) Add 1.5 ml of Reagent A to all sample tubes, calibrators, and control.

(2) Add 50 microliters (µl) of Reagent B to all sample tubes, calibrators, and control. Cap the tubes and mix contents with a vortex for 10 seconds.

(3) Heat the tubes in a 50°C bath for 10 minutes.

(4) Remove tubes from the bath and cool to room temperature. Read the samples in the fluorometer within 1 hour.

Note: Cuvettes may be placed in tap water for 30 seconds to cool. Dry the outside wall of the cuvette completely before placing in the fluorometer.

e. Fluorometer Reading.

Calibrate the fluorometer using the following procedure:

(1) Turn on the power (no warm-up is necessary).

(2) Change the date or time - If correct, press the "Continue" key.

(3) When asked for Test Delay Time, enter "2" and press the "Enter" key.

(4) When asked for answer format, select "Decimals."

(5) When asked for measurement units, select "ppm."

(6) At the "insert red vial" prompt, place the appropriate calibrator cuvette into the sample well.

(7) When asked for the calibrator value, enter the appropriate value (refer to the card supplied with the calibrators for the red value) and press the "Enter" key.

(8) When asked to "remove the red vial," remove the calibrator tube from the sample well.

(9) At the "insert green vial" prompt, place the appropriate calibrator cuvette into the sample well.

(10) When asked for the "blank value, "enter the appropriate value (refer to the card supplied with the calibrators for the green value) and press the "Enter" key.

(11) When asked to "remove the green vial," remove the calibrator tube from the sample well.

(12) At the "insert test vial" prompt, place the control cuvette into the sample well.

(13) The fluorometer will now display the value for the control vial.

(14) Compare the value of the control with the values listed on the card. If the control value is within the specified range, the fluorometer is ready to analyze samples. If the value is outside of the specified range, rerun the red, green, and yellow calibration cuvettes. If the control value still exceeds the specified range limit, contact Vicam.

(15) Press the "Enter" key. The fluorometer is now ready to analyze samples.

f. Reading the Results.

To determine the DON concentration insert the cuvette containing the sample portion into the sample well of the fluorometer. The DON concentration will appear on the display after the appropriate 2-second delay. Read the results.

12.4 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

12.5 SUPPLEMENTAL ANALYSIS

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (acetonitrile/water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the conformance limit of the test kit.

12.6 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

12.7 WASTE DISPOSAL

Transfer sample extract solutions (acetonitrile/water) and derivatization solutions into a liquid waste container for disposal. Follow SOP, established by the field office, for handling and disposing of hazardous waste.

Transfer sample slurry, used filter paper, cuvettes, caps, and columns into the normal solid waste container for routine disposal.

12.8 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) Glass culture tubes (15 x 85 mm) - 25 tubes per test kit

(2) DON FQ (or DON FQ MB for malted barley) columns

(3) Glass cuvettes and caps (12 x 75 mm) - 50 per test kit

(4) Reagent A - Ethylenediamine in methanol

(5) Reagent B - Zirconyl Nitrate in methanol

(6) Calibrator solutions (red-label, and green-label) plus control solution (yellow-label)

b. Materials Required but not Provided:

(1) Blender and blender jars. The unit must be explosion proof.

(2) Funnel

(3) Beakers - 250 ml

(4) 250 ml graduated cylinder

(5) Extraction Solvent - Acetonitrile/distilled or deionized water (84/16)

(6) Filter paper - Vicam part # 31240, Whatman No. 1, or equivalent

(7) Carboy - 2 gallon capacity

(8) Vortex mixer

(9) Pipette and tips - 50 µl

(10) Pipette and tips - 1.5 ml

(11) Fluorometer with printer - Romer RL 100, Vicam Series III and IV, Torbex 100, Vicam FX-100)

(12) Vacuum Pump and Trap

(13) E-Vap Evaporator

(14) Test Tube Rack

(15) Thermometer

(16) Dry Bath with Heating Block

(17) Sample grinder

(18) Balance

12.9 STORAGE CONDITIONS

a. Columns.

DON FQ and DON FQ MB columns - Room temperature in a drawer or box.

b. Reagents.

Reagents A & B are shipped in amber bottles, cap tightly and store in a temperature controlled area (between 40° and 80° F). Do not freeze. Reagents should stay stable for 6 months.

c. Calibration and Control Solutions.

Calibration and control solutions are shipped in amber vials, cap tightly and store in the refrigerator. Solutions should stay stable for 6 months.